Analytical Results of Alpha-tocopherol for Dietary Supplement Ingredient Database in Adult and Children's Multivitamin Studies

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DSID Dietary Supplement Ingredient Database A U.S. Government Initiative

USDA

NIH National Institutes of Health Office of Dietary Supplements

Introduction

The majority of adults in the United States take one or more dietary supplements in the form of multivitamins, to improve or maintain their overall health [1]. Data collected from the National Health and Nutrition Examination Survey (NHANES) among adult users and non-users have shown that supplement users have higher mean vitamin intakes for some vitamins (folic acid, vitamins A and E) from foods than non-supplement users [2]. Vitamin E is widely known for its health benefits and immune enhancement. Free radicals react rapidly with oxygen to form reactive oxygen species (ROS) that damage cells and can cause chronic diseases over time. The antioxidant properties of vitamin E protect cells from the damaging effects of free radicals by inhibiting production of ROS and strengthening the immune system [3]. Vitamin E is a group of fat-soluble compounds, found naturally in many foods and available in dietary supplements. The eight forms of vitamin E exist as tocopherols and tocotrienols; all have varying levels of biological activity. The most biologically active form is alpha-tocopherol, the only form maintained in the plasma. Many multivitamin/mineral (MVM) products sold in the US contain alpha-tocopherol, added as the natural form (*RRR*-alpha-tocopherol) labeled as "d" or the synthetic form (all-*rac*-alpha-tocopherol) which is labeled as "dl". The form of vitamin E is important since the labeled level is reported in International Units (IU) and the conversion factor for each differs when calculating analytical values and comparing them to labeled level values. The Recommended Dietary Allowances (RDA) developed by the Food and Nutrition Board (FNB) for natural alpha-tocopherol is 22.4 IU for 14+ years, 10.4 IU for children 4-8 years, 16.4 IU for children 9-13 years. The daily value (100%DV) for natural alpha-tocopherol is 30 IU for both adults and children over 4 years.

Objective

Vitamin E and 19 other vitamins and minerals were chemically analyzed and evaluated as part of the Dietary Supplement Ingredient Database (DSID) project, funded by the NIH Office of Dietary Supplements (ODS) and collaboratively administered with the US Department of Agriculture at the Beltsville Agricultural Research Center (BARC). Products were purchased based on a statistical national sampling plan and the analytical values were evaluated. The objective was to publish national estimates of ingredients in adult and children's MVM (≥ 3 vitamins plus mineral) products which were reported in the DSID-2, released in March 2012. Researchers can utilize these estimates in studying the health impacts of food and supplement intake.

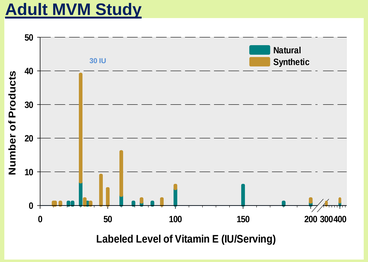
Dietary Supplement Ingredient Database (DSID) Website:
http://dietarysupplementdatabase.usda.nih.gov/

Sampling Plan

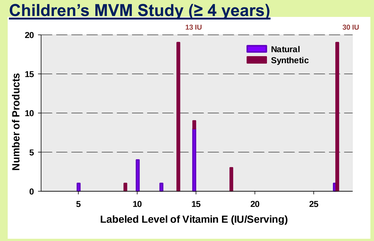
For both MVM studies, data were collected from NHANES and other sources, including the Nutrition Business Journal (NBJ) and the Natural Marketing Institute (NMI), to identify representative products and develop statistical national sampling plans. Products were purchased from three market channels, mass merchandisers, natural/health food stores, and direct sales (internet). For each product, multiple lots were obtained and the information on the label such as ingredient source, level, and percent daily value (%DV) were recorded in the database. For the adult MVMs, 86 out of 115 products contained vitamin E. Of these, 28 products listed the natural form of vitamin E (*RRR*-alpha-tocopherol) while 58 had the synthetic form (all-*rac*-alpha-tocopherol). For the children's MVM study, 62 out of 65 product contained vitamin E and the breakdown for natural or synthetic vitamin E is 18 and 44, respectively. Figure 1 shows the range of labeled levels obtained from the supplement facts panel of all products. The most common level for adult MVMs was 30 IU. For children's MVMs (serving size ≥ 4 years), both 13 IU and 30 IU were commonly labeled levels.

Fig.1 Range of Labeled Levels for alpha-tocopherol in MVM products

Adult MVM Study



Children's MVM Study (≥ 4 years)



Method of Analysis and QC

A quality assurance and control plan was implemented to monitor the accuracy and precision of the analytical results from contracted laboratories. The quality control materials used were NIST (National Institute of Standards and Technology) Standard Reference Material (SRM) 3280 and two or three in-house control materials, which are a single lot of a MVM product purchased in bulk. In both the adult and children's MVM studies, QC materials were included in every batch of 15-20 samples. Each sample contained at least 20 units of tablets, capsules, or gummies. For powder and liquid samples, a comparable amount of material was weighed out and sent for analysis to the contracted laboratories.

The following was included in a typical batch:

- NIST SRM3280, MVM matrix (1)
- A set of product duplicates (two sets of 20 units of the same MVM product with different test sample IDs)
- Two to three samples of in-house control materials
- Product samples (15)

At the lab, each sample was homogenized, treated with enzyme and saponified with KOH. The sample was then extracted with organic solvent, such as hexane, then followed by quantification on an HPLC silica column using fluorescence detection.

Fig.2 Alpha-tocopherol Results of NIST SRM 3280 for both studies

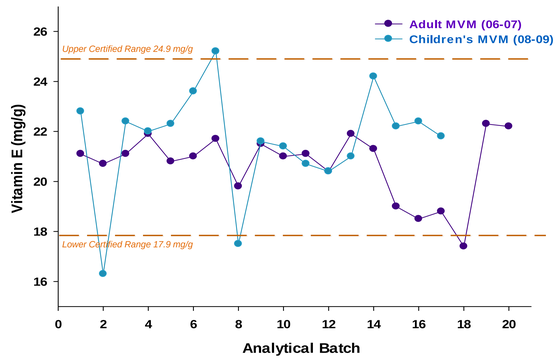


Fig.3 Analytical Results for α-tocopherol in Adult MVM Products: Mean % Difference from Labeled Level

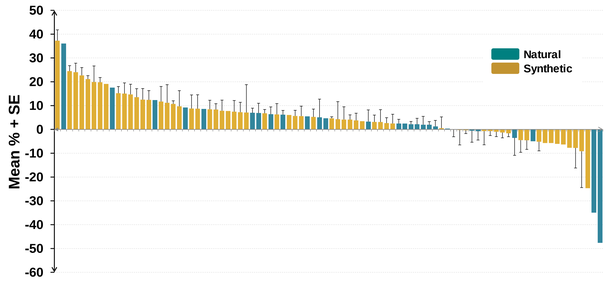


Fig.4 Analytical Results for α-tocopherol in Children's MVM Products: Mean % Difference from Labeled Level

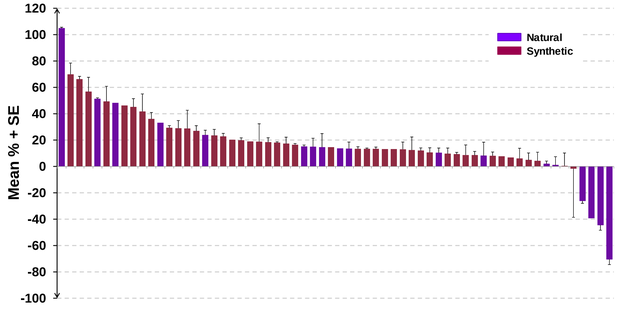


Table 1. NIST Control Data

SRM	Study	Lab Mean (mg/g)	Certified Value (mg/g)	Certified Value Acceptable Range	Percent Difference from Certified Value	*n*	RSD
NIST 3280	Adult MVM	20.45	21.4	± 3.5 mg/g	- 4.44%	21	8.06
NIST 3280	Children's MVM	21.64	21.4	± 3.5 mg/g	+ 1.10%	17	10.01

Results & Conclusions

The first step in reviewing laboratory data is to assess the quality control results, gathered from all analytical batches. Table 1 summarizes the lab analytical mean of the SRM results, relative standard deviation (RSD), and percent difference from certified value. Figure 2 shows the NIST SRM 3280 analytical results (mg/g) from 20 batches of samples from the adult MVM study (86 products) and 17 batches from the children's MVM study (62 products). The upper and lower certified ranges are identified on the graph. The results for all but one batch fell within this range in the adult MVM study and all but three batches from the children's MVM study. The mean of 20.45 mg/g for adult MVMs and 21.64 mg/g for children's MVMs compared to the certified NIST value of 21.4 mg/g with a RSD of 8.06% and 10.01% respectively, were both considered acceptable. Batches with QC results out of range were reviewed carefully and identified as questionable with either low or high bias. The product results in those batches were evaluated critically and were more likely to be retested. Product results showing unusually high or low results compared to labeled levels or high variability among product lots were identified for retesting, even if QC data for these batches were acceptable.

Once the laboratory results for samples were finalized for each study, they were compared to the labeled levels for each product based on the form of vitamin E. Since the label provides the amount in IU, the conversion of vitamin E from IU to mg was necessary (IU*0.67=mg, for natural, IU*0.9=mg for synthetic). A percent difference from label was then calculated. Figures 3 and 4 show the analytical results for the products in each study calculated as mean percent difference from label with standard error (SE). Over 70% of products in the adult MVM study and 91% of children's MVM products were above the labeled level. In the adult MVM study, the mean percent difference from label was 4.3% for natural α-tocopherol and 5.7% for synthetic. The children's MVM study mean percent difference from label was 18% for natural and 21% for synthetic.

Additional statistical evaluation of this data determined national estimates based on regression predictions across the range of labeled levels. The predicted mean percent difference from label at the most common labeled level (30 IU) for the adult MVM study was 6.0 ± 1.1% (Mean + SE). In children's MVM study, the predicted mean percent difference from label at the most common labeled level at 13 IU was 22 ± 4.4% and at 30 IU was 14 ± 3.9%.

Further Research

- DSID has begin a monitoring study of adult MVMs, which focuses on analyzing representative adult MVM products for specific nutrients of interests such as iodine, vitamins A and D, and chromium. NDL is currently processing and reviewing analytical data received from initial and retest samples.

- The next release of DSID (DSID-3) is planned for late 2014. This release will report on the vitamin and mineral content of over-the-counter prenatal MVM products and the levels of EPA, DHA, and ALA in omega-3 supplements.

References:

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